PB82-117540

Recommended Guideline for Acute Dermal Toxicity Test

(U.S.) Interagency Regulatory Liaison Group
Washington, DC

Jan 81

U.S. Department of Commerce
National Technical Information Service
NTIS

PB82-117540

January 1981

Interagency Regulatory Liaison Group

TESTING STANDARDS AND GUIDELINES WORK GROUP

Recommended Guideline For

ACUTE DERMAL TOXICITY TEST

REPRODUCED BY
NATIONAL TECHNICAL INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

U.S. Consumer Product Safety Commission
U.S. Environmental Protection Agency
Food and Drug Administration (U.S. Dept. of HHS)
Food Safety and Quality Service (U.S. Dept. of Agriculture)
Occupational Safety and Health Administration (U.S. Dept. of Labor)

FOREWORD

Four regulatory agencies, the Consumer Product Safety Commission **(CPSC)**, the Environmental Protection Agency **(EPA)**, the Food and Drug Administration **(FDA)**, and the Occupational Safety and Health Administration **(OSHA)**, agreed to work together to reform the regulatory process and to improve protection of workers, public health, and the environment **(42 FR 54856, 11 October 1977)**. They formed the Interagency Regulatory Liaison Group **(IRLG)** to implement their agreement. In January 1979, the Food Safety and Quality Service **(FSQS)**, Department of Agriculture, joined the IRLG.

These agencies recognized that although they often regulate the same chemicals, toxicity testing guidelines used by each agency are not always uniform. Among currently required tests, differences exist primarily in details of methodology and not in fundamental toxicological principles. The Testing Standards and Guidelines Work Group was established for the purpose of developing guidelines which would resolve existing differences and be used by all of the IRLG agencies for testing chemicals for health or environmental effects or both. This effort was to be coordinated with the development of other guidelines. On December 17, 1977, the Work Group held a public meeting to explain its purpose and goals, and to answer questions about its activities **(42 FR 59106, 15 November 1977)**.

IRLG guidelines are derived from those currently used or under development in the agencies, in international organizations, and in industry. Early drafts were circulated through each agency for review, and comments from that review are included in this guideline. In addition, drafts of the EPA Office of Pesticides Programs **(OPP)** proposed guidelines **(43 FR 37336, 22 August 1978)** under the Federal Insecticide, Fungicide and Rodenticide Act **(FIFRA)**, and public comments to those guidelines were used extensively in an effort to assure compatibility and to benefit from the appreciable time and effort the OPP staff put into their development. A second major source of information was *Principles and Procedures for Evaluating the Toxicity of Household Substances* **(NAS Pub. No. 1138)**, prepared for CPSC by the National Academy of Sciences. Still others heavily relied on were the Pharmaceutical Manufacturers' Association *Guidelines for the Assessment of Drug and Medical Device Safety in Animals* **(February 1977)**; the FDA, Bureau of Foods Direct Additive Cyclic Review Draft Guidelines; the *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics*, Association of Food and Drug Officials of the U. S.; and protocols submitted by several industrial organizations in response to **43 FR 1987, 13 January 78**. The Work Group also used written and verbal comments received from the public in response to **44 FR 49015, 21 August 1979**. IRLG guidelines are being coordinated with development of guidelines by the Organization for Economic Cooperation and Development **(OECD)**. The Work Group acknowledges the contributions from each of these information sources and takes this opportunity to express its appreciation to the scientists who developed them and made them available.

Under most circumstances, the data obtained from this test should be sufficient to meet the requirements for a specific aspect of the toxicological characterization of the test substance. Under certain circumstances, however, the data may show a need for further study; or it may be recognized at the outset this guideline may have to be modified by an agency. It is important that tests be agreed upon between the investigator and the agency requiring them.

Each agency has the responsibility to decide what substance, and what form of the substance, it requires to be tested; which tests it will require; and how it will use the data derived from the tests.

INTRODUCTION

A test for acute dermal toxicity should evaluate the potential for systemic and local toxic effects of chemicals expected to come in contact with the skin. The acute dermal test refers to one period of topical application of up to 24 hours (the exposure period) and an observation period of at least 14 days.

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I. General Considerations

A. Good Laboratory Practices

Studies should be conducted according to good laboratory practice regulations (*e.g.*, "Nonclinical Laboratory Studies, Good Laboratory Practice Regulations," 43 FR 59986, 22 December 1978).

B. Test Substance

1. The specific substance or mixture of substances to be tested should be determined in consultation with the responsible agency. As far as is practical, composition of the test substance should be known. Information should include the name and quantities of all major components, known contaminants and impurities, and the percentage of unidentifiable materials, if any, to account for 100% of the test sample.

2. Ideally, the lot of the substance tested should be the same throughout the study. The test sample should be stored under conditions that maintain its stability, strength, quality, and purity from the date of its production until the tests are complete.

C. Animals

1. Healthy animals, not subjected to any previous experimental procedures, must be used.

2. The test animal shall be characterized as to species, strain, sex, weight and/or age. Each animal must be assigned an appropriate identification number.

3. Recommendations contained in DHEW pub. no. (NIH) 74-23, entitled '*Guide for the Care and Use of Laboratory Animals*,' should be followed for the care, maintenance, and housing of animals.

4. Animals should be caged individually for this test.

5. Animals must be assigned to groups in such a manner as to minimize bias and assure comparability of pertinent variables (for example, weight variation in animals used in a test should not exceed ± 20% of the mean weight).

D. Dead Animals, Necropsy, and Histopathology

Animals should be observed as necessary to insure that there is minimal loss due to cannibalism or management problems. Where possible, necropsy must be performed soon after an animal is sacrificed or found dead to minimize loss of tissues due to autolysis. When necropsy cannot be performed immediately, the animal must be refrigerated at temperatures low enough to minimize autolysis. If histopathological examination is to be conducted, tissue specimens should be placed in appropriate fixative when they are taken from the animal.

II. Specific Considerations

A. Test Preparation

1. Animals: The rat, rabbit or guinea pig may be used; however, the young, adult, albino rabbit weighing 2.0 to 3.0 kg is the preferred species because of its size, ease of handling and restraint, and skin permeability. If rats or guinea pigs are used, rats weighing 200 to 300 g or guinea pigs weighing 350 to 450 g are suggested. Selection of other species may be acceptable but must be justified.

2. Preparation of skin: Approximately twenty-four hours before testing, fur from the back of animals should be clipped so that no less than 10% (about 240 cm^2) of the total body surface area is available for application of material.

3. Limit test: A trial test on abraded skin is recommended. The abraded area may be prepared by making four epidermal incisions with a clean needle through the stratum corneum (not deep enough to disturb the derma or produce bleeding), but other acceptable methods may be used. If a test at a dose of 2 g/kg body weight (or 2 ml/kg) or more, using the procedures described for this study, produces no compound related mortality, then a full study using 3 dose levels may not be necessary.

4. Number and sex: Equal numbers of animals of each sex with intact skin are required for each dose level. The number of animals per dose depends on the level of statistical confidence desired. Five animals per sex per dose are recommended in most cases. Females should be nulliparous and nonpregnant.

5. Dose levels: If mortality occurs in the limit test, an additional test should be conducted using rabbits with intact skin. At least three dose levels spaced appropriately to produce test groups with a range of toxic effects and mortality rates should be used.

B. Test Procedure

1. Test substance: Liquids should be tested directly, but solid test substances should be moistened sufficiently with normal saline or tap water to make a paste that will insure good contact with the skin. For some applications, it may be appropriate or necessary to use other vehicles. If a carrier or diluent is used, it should be non-irritating and of known low toxicity. When such vehicles are used, consideration should be given to the effects of those vehicles on absorption of the test substance.

2. Dosage: When technically feasible, the maximum quantity of substance plus vehicle to be applied is 2 g/kg body weight. The test substance should be applied uniformly over the dorsal surface area.

3. Administration (application): The test substance must remain in contact with the skin throughout the exposure period of 24 hours. Liquid or solid substances should be held in contact with the skin with a porous gauze dressing and non-irritating tape. The test site should be covered with an impermeable material such as plastic film or rubberized cloth in a semi-occlusive fashion, allowing air to pass between the skin and the covering. Occlusive skin dressings may enhance penetration of the test substance and should be used only when testing for effects that may occur under similar conditions in humans.

During exposure, animals should be prevented from ingesting or inhaling the test substance. Restrainers, such as Elizabethan collars, that permit animals to move about their cages should be used for this purpose. Immobilization is not recommended.

At the end of the exposure period, all residual material should be removed by washing, using an appropriate solvent. About one half hour later, and once again at 72 hours, the exposed area should be examined, and all lesions noted and graded (Table I).

4. Observation period: The observation period must be at least 14 days. Although a 14-day observation period is sufficient for most compounds, animals demonstrating visible signs of toxicity at 14 days may be held longer.

5. Clinical observations: A careful clinical observation of each animal should be made at least once a day. Additional observations should be made daily with appropriate actions taken to minimize loss of animals to the study, *e.g.*, necropsy or refrigeration of those animals found dead and isolation or sacrifice of weak or moribund animals. All toxicological and pharmacological signs should be recorded including time of onset, intensity, and duration. The time of death should also be noted. Individual records should be maintained for each animal.

6. Weight change: Animals must be weighed individually on the day the test substance is administered, weekly thereafter, and at death or sacrifice.

7. Necropsy: A complete gross necropsy should be performed on all animals that die during the course of the test. Consideration should be given to gross necropsy of the animals sacrificed at termination of the test. Microscopic examination of gross lesions should also be considered. If the substance will not be subjected to additional acute or multiple dose testing that includes gross necropsy, or if the results of this test are to be used for labeling purposes, complete gross necropsy should be performed on all remaining animals at termination of the test.

TABLE I

EVALUATION OF SKIN REACTION

	Value
Erythema and Eschar Formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness to slight eschar formation (injuries in depth)	4
Edema Formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond the area of exposure	4
Severe eschar and/or corrosion	Note occurrence

III. Data Reporting

A. Identification

Each test report should be signed by the persons responsible for the test and identify:

1. The laboratory where the test was performed by name and address;

2. The inclusive dates of the test; and

3. Each person primarily responsible for separate components of the test including (a) the conduct of the test, (b) pathology, (c) analysis of the data, (d) the writing of the report, and (e) any written or other matter contained in the report.

B. Body of Report

The test report must include all information necessary to provide a complete and accurate description and evaluation of the test procedures and results, in the following sections:

1. Summary and conclusions: This section of the test report should contain a brief description of the methods, a summary of the data, an analysis of the data, and a statement of the conclusions drawn. The summary must highlight all positive data or observations and any deviations from control data which may be indicative of toxic effects.

2. Materials: This section of the test report should include, but not be limited to, the following information:

   (a) Identification of the test substance, so far as practical, including:

      i. chemical name, molecular structure, and a qualitative and quantitative determination of its chemical composition, including names and quantities of known contaminants and impurities and listing the percentage of unidentifiable materials to account for 100% of the test sample.

      ii. manufacturer and lot number of the substance tested, and such information as physical state, pH, stability, and purity; and

      iii. specific identification of diluents, suspending agents, emulsifiers, or other materials used in administering the test substance.

   (b) Animal data, including:

      i. species and strain used and rationale for selection of the strain if other than a common laboratory strain;

      ii. source of supply of the animals;

      iii. description of any pre-test conditioning;

iv. description of the method used in randomization of animals to test or control groups; and

v. numbers, age, and condition of animals of each sex in each test and control group.

(c) Data on husbandry should include description of the caging conditions including number of animals per cage, diet, bedding material, ambient temperature, humidity, and lighting conditions.

3. Methods:

(a) Deviation from guidelines - This section shall indicate all ways in which the test procedure deviates from these guidelines and shall state the rationale for such deviation.

(b) Specification of test methods - This section shall include a full description of the experimental design and procedure, the length of the study, and the dates on which the study began and ended.

(c) Statistical analysis - All statistical methods used should be fully described or identified by reference.

(d) Data on dosage administration, including:

i. all dose levels administered, expressed as mg/kg of body weight;

ii. method and frequency of administration; and

iii. total volume of substance (*i.e.*, test substance plus vehicle) contained in individual dosages.

(e) Data on observation methods, including:

i. duration; and

ii. method and frequency of observation of the animals.

4. Results:

The tabulation of data and individual results must accompany each report in sufficient detail to permit independent evaluation of results, including summaries and tables that show, as appropriate, the relationship of effects to time of dosing, sex, etc.

IV. Suggested Reading

1. Draize, J. H. 1959. Dermal toxicity. *In*: Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics Austin, Texas. Association of Food and Drug Officials of the U.S. pp. 46-59.

2. National Academy of Sciences - National Research Council, 1977. Dermal and eye toxicity tests. *In*: Principles and Procedures for Evaluating the Toxicity of Household Substances, Report No. 1138, prepared for the Consumer Product Safety Commission. pp. 23-28.

3. McCreesh, A H. and M. Steinberg, 1977. Dermato -Toxicology and Pharmacology, Hemisphere Publishing Corp., Washington, D.C.

4. Mailbach, H. I. and F. N. Marzulli. 1975. Animal Models in Dermatology. Churchill and Livingston, Edinburgh.

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The IRLG Testing Standards and Guidelines Work Group dedicates its guidelines to the memory of Dr. William D'Aguanno, whose commitment and talent contributed immeasurably to their development.